BIOMARKERS AND METHODS FOR DETERMINING SENSITIVITY TO EPIDERMAL GROWTH FACTOR RECEPTOR MODULATORS

FIELD OF THE INVENTION

The present invention relates generally to the field of pharmacogenomics, and more specifically to methods and procedures to determine sensitivity in patients to allow the development of individualized genetic profiles which aid in treating diseases and disorders based on patient response at a molecular level.

BACKGROUND OF THE INVENTION:

Cancer is a disease with extensive histoclinical heterogeneity. Although conventional histological and clinical features have been correlated to prognosis, the same apparent prognostic type of tumors varies widely in its responsiveness to therapy and consequent survival of the patient.

New prognostic and predictive markers, which would facilitate an individualization of therapy for each patient, are needed to accurately predict patient response to treatments, such as small molecule or biological molecule drugs, in the clinic. The problem may be solved by the identification of new parameters that could better predict the patient's sensitivity to treatment. The classification of patient samples is a crucial aspect of cancer diagnosis and treatment. The association of a patient's response to a treatment with molecular and genetic markers can open up new opportunities for treatment development in non-responding patients, or distinguish a treatment's indication among other treatment choices because of higher confidence in the efficacy. Further, the pre-selection of patients who are likely to respond well to a medicine, drug, or combination therapy may reduce the number of patients needed in a clinical study or accelerate the time needed to complete a clinical development program (M. Cockett et al., 2000, *Current Opinion in Biotechnology*, 11:602-609).

The ability to predict drug sensitivity in patients is particularly challenging because drug responses reflect not only properties intrinsic to the target cells, but also a host's metabolic properties. Efforts to use genetic information to predict drug sensitivity have primarily focused on individual genes that have broad effects, such as the multidrug resistance genes, *mdr1* and *mrp1* (P. Sonneveld, 2000, *J. Intern. Med.*, 247:521-534).

The development of microarray technologies for large scale characterization of gene mRNA expression pattern has made it possible to systematically search for molecular markers and to categorize cancers into distinct subgroups not evident by traditional histopathological methods (J. Khan et al., 1998, *Cancer Res.*, 58:5009-5013; A.A. Alizadeh et al., 2000, *Nature*, 403:503-511; M. Bittner et al., 2000, *Nature*, 406:536-540; J. Khan et al., 2001, *Nature Medicine*, 7(6):673-679; and T.R. Golub et al., 1999, *Science*, 286:531-537; U. Alon et al., 1999, *Proc. Natl. Acad. Sci. USA*, 96:6745-6750). Such technologies and molecular tools have made it possible to monitor the expression level of a large number of transcripts within a cell population at any given time (see, e.g., Schena et al., 1995, *Science*, 270:467-470; Lockhart et al., 1996, *Nature Biotechnology*, 14:1675-1680; Blanchard et al., 1996, *Nature Biotechnology*, 14:1649; U.S. Patent No. 5,569,588 to Ashby et al.).

Recent studies demonstrate that gene expression information generated by microarray analysis of human tumors can predict clinical outcome (L.J. van't Veer et al., 2002, *Nature*, 415:530-536; M. West et al., 2001, *Proc. Natl. Acad. Sci. USA*, 98:11462-11467; T. Sorlie et al., 2001, *Proc. Natl. Acad. Sci. USA*, 98:10869-10874; M. Shipp et al., 2002, *Nature Medicine*, 8(1):68-74). These findings bring hope that cancer treatment will be vastly improved by better predicting the response of individual tumors to therapy.

Needed are new and alternative methods and procedures to determine drug sensitivity in patients to allow the development of individualized genetic profiles which are necessary to treat diseases and disorders based on patient response at a molecular level.

SUMMARY OF THE INVENTION:

The invention provides methods and procedures for determining patient sensitivity to one or more Epidermal Growth Factor Receptor (EGFR) modulators. The invention also provides methods of determining or predicting whether an individual requiring therapy for a disease state such as cancer will or will not respond to treatment, prior to administration of the treatment, wherein the treatment comprises one or more EGFR modulators. The one or more EGFR modulators are compounds that can be selected from, for example, one or more EGFR specific ligands, one or

more small molecule EGFR inhibitors, or one or more EGFR binding monoclonal antibodies.

In one aspect, the invention provides a method for identifying a mammal that will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises: (a) measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1; (b) exposing the mammal to the EGFR modulator; (c) following the exposing of step (b), measuring in the mammal the level of the at least one biomarker, wherein a difference in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates that the mammal will respond therapeutically to said method of treating cancer.

As used herein, respond therapeutically refers to the alleviation or abrogation of the cancer. This means that the life expectancy of an individual affected with the cancer will be increased or that one or more of the symptoms of the cancer will be reduced or ameliorated. The term encompasses a reduction in cancerous cell growth or tumor volume. Whether a mammal responds therapeutically can be measured by many methods well known in the art, such as PET imaging.

The mammal can be, for example, a human, rat, mouse, dog rabbit, pig sheep, cow, horse, cat, primate, or monkey.

The method of the invention can be, for example, an in vitro method and wherein the at least one biomarker is measured in at least one mammalian biological sample from the mammal. The biological sample can comprise, for example, at least one of whole fresh blood, peripheral blood mononuclear cells, frozen whole blood, fresh plasma, frozen plasma, urine, saliva, skin, hair follicle, or tumor tissue.

In another aspect, the invention provides a method for identifying a mammal that will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises: (a) exposing the mammal to the EGFR modulator; (b) following the exposing of step (a), measuring in the mammal the level of the at least one biomarker selected from the biomarkers of Table 1, wherein a difference in the level of the at least one biomarker measured in step (b), compared to the level of the biomarker in a mammal that has not been

exposed to said EGFR modulator, indicates that the mammal will respond therapeutically to said method of treating cancer.

In yet another aspect, the invention provides a method for testing or predicting whether a mammal will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises: (a) measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1; (b) exposing the mammal to the EGFR modulator; (c) following the exposing of step (b), measuring in the mammal the level of the at least one biomarker, wherein a difference in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates that the mammal will respond therapeutically to said method of treating cancer.

In another aspect, the invention provides a method for determining whether a compound inhibits EGFR activity in a mammal, comprising: (a) exposing the mammal to the compound; and (b) following the exposing of step (a), measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1, wherein a difference in the level of said biomarker measured in step (b), compared to the level of the biomarker in a mammal that has not been exposed to said compound, indicates that the compound inhibits EGFR activity in the mammal.

In yet another aspect, the invention provides a method for determining whether a mammal has been exposed to a compound that inhibits EGFR activity, comprising (a) exposing the mammal to the compound; and (b) following the exposing of step (a), measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1, wherein a difference in the level of said biomarker measured in step (b), compared to the level of the biomarker in a mammal that has not been exposed to said compound, indicates that the mammal has been exposed to a compound that inhibits EGFR activity.

In another aspect, the invention provides a method for determining whether a mammal is responding to a compound that inhibits EGFR activity, comprising (a) exposing the mammal to the compound; and (b) following the exposing of step (a), measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1, wherein a difference in the level of said biomarker measured

in step (b), compared to the level of the biomarker in a mammal that has not been exposed to said compound, indicates that the mammal is responding to the compound that inhibits EGFR activity.

As used herein, "responding" encompasses responding by way of a biological and cellular response, as well as a clinical response (such as improved symptoms, a therapeutic effect, or an adverse event), in a mammal

The invention also provides an isolated biomarker selected from the biomarkers of Table 1. The biomarkers of the invention comprise sequences selected from the nucleotide and amino acid sequences provided in Table 1 and the Sequence Listing, as well as fragments and variants thereof.

The invention also provides a biomarker set comprising two or more biomarkers selected from the biomarkers of Table 1.

The invention also provides kits for determining or predicting whether a patient would be susceptible or resistant to a treatment that comprises one or more EGFR modulators. The patient may have a cancer or tumor such as, for example, a colon cancer or tumor.

In one aspect, the kit comprises a suitable container that comprises one or more specialized microarrays of the invention, one or more EGFR modulators for use in testing cells from patient tissue specimens or patient samples, and instructions for use. The kit may further comprise reagents or materials for monitoring the expression of a biomarker set at the level of mRNA or protein.

In another aspect, the invention provides a kit comprising two or more biomarkers selected from the biomarkers of Table 1.

In yet another aspect, the invention provides a kit comprising at least one of an antibody and a nucleic acid for detecting the presence of at least one of the biomarkers selected from the biomarkers of Table 1. In one aspect, the kit further comprises instructions for determining whether or not a mammal will respond therapeutically to a method of treating cancer comprising administering a compound that inhibits EGFR activity. In another aspect, the instructions comprise the steps of (a) measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1, (b) exposing the mammal to the compound, (c) following the exposing of step (b), measuring in the mammal the level of the at least one biomarker,

wherein a difference in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates that the mammal will respond therapeutically to said method of treating cancer.

The invention also provides screening assays for determining if a patient will be susceptible or resistant to treatment with one or more EGFR modulators.

The invention also provides a method of monitoring the treatment of a patient having a disease treatable by one or more EGFR modulators.

The invention also provides individualized genetic profiles which are necessary to treat diseases and disorders based on patient response at a molecular level.

The invention also provides specialized microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, comprising one or more biomarkers having expression profiles that correlate with either sensitivity or resistance to one or more EGFR modulators.

The invention also provides antibodies, including polyclonal or monoclonal, directed against one or more biomarkers of the invention.

The invention will be better understood upon a reading of the detailed description of the invention when considered in connection with the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES:

FIG. 1 illustrates the gene filtering process.

FIG. 2 illustrates the cell line filtering process.

FIG. 3 illustrates the cell line IC50 data.

FIG. 4 illustrates the T-test Results I.

FIG. 5 illustrates the T-test Results II.

FIG. 6 illustrates the T-test Results III.

DETAILED DESCRIPTION OF THE INVENTION:

The invention provides biomarkers that respond to the modulation of a specific signal transduction pathway and also correlate with EGFR modulator sensitivity or resistance. These biomarkers can be employed for predicting response

to one or more EGFR modulators. In one aspect, the biomarkers of the invention are those provided in Table 1 and the Sequence Listing, including both polynucleotide and polypeptide sequences.

TABLE 1 - BIOMARKERS

Unigene title and SEQ ID NOS:	Affymetrix Description	Affymetrix Probe Set
Cadherin 17, LI cadherin (liver-intestine) SEQ ID NOS:1 (nucleotide) and 67 (amino acid)	gb:U07969.1 /DEF=Human intestinal peptide-associated transporter HPT-1 mRNA, complete cds. /FEA=mRNA /PROD=intestinal peptide-associated transporter HPT-1 /DB_XREF=gi:483391 /UG=Hs.89436 cadherin 17, LI cadherin (liver-intestine) /FL=gb:NM_004063.1 gb:U07969.1	209847_at
Carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen) SEQ ID NOS:2 (nucleotide) and 68 (amino acid)	gb:BC005008.1 /DEF=Homo sapiens, carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen), clone MGC:10467, mRNA, complete cds. /FEA=mRNA /PROD=carcinoembryonic antigen-related cell adhesionmolecule 6 (non-specific cross reacting antigen) /DB_XREF=gi:13477106 /UG=Hs.73848 carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen) /FL=gb:BC005008.1 gb:M18216.1 gb:M29541.1 gb:NM_002483.1	203757_s_at
Carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen) SEQ ID NOS:3 (nucleotide) and 69 (amino acid)	gb:M18728.1 /DEF=Human nonspecific crossreacting antigen mRNA, complete cds. /FEA=mRNA /GEN=NCA; NCA; NCA /PROD=non-specific cross reacting antigen /DB_XREF=gi:189084 /FL=gb:M18728.1	211657_at
Lectin, galactoside-binding, soluble, 1 (galectin 1) SEQ ID NOS:4 (nucleotide) and 70 (amino acid)	gb:NM_002305.2 /DEF=Homo sapiens lectin, galactoside-binding, soluble, 1 (galectin 1) (LGALS1), mRNA. /FEA=mRNA /GEN=LGALS1 /PROD=beta-galactosidase binding lectin precursor /DB_XREF=gi:6006015 /UG=Hs.227751 lectin, galactoside-binding, soluble, 1 (galectin 1)	201105_at

	/FL=gb:BC001693.1 gb:J04456.1 gb:NM_002305.2	
Transmembrane protease, serine 2 SEQ ID NOS:5 (nucleotide) and 71 (amino acid)	gb:AF270487.1 /DEF=Homo sapiens androgen-regulated serine protease TMPRSS2 precursor (TMPRSS2) mRNA, complete cds. /FEA=mRNA /GEN=TMPRSS2 /PROD=androgen-regulated serine protease TMPRSS2precursor /DB_XREF=gi:13540003 /FL=gb:AF270487.1	211689_s_at
Mucin 5, subtypes A and C, tracheobronchial/gastric SEQ ID NOS:6 (nucleotide), 7 (nucleotide) and 72 (amino acid)	Consensus includes gb:AW192795 /FEA=EST /DB_XREF=gi:6471494 /DB_XREF=est:xl51d08.x1 /CLONE=IMAGE:2678223 /UG=Hs.103707 apomucin	214303_x_at
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial) SEQ ID NOS:8 (nucleotide) and 73 (amino acid)	gb:NM_005518.1 /DEF=Homo sapiens 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial) (HMGCS2), mRNA. /FEA=mRNA /GEN=HMGCS2 /PROD=3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2(mitochondrial) /DB_XREF=gi:5031750 /UG=Hs.59889 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial) /FL=gb:NM_005518.1	204607_at
Interferon-stimulated protein, 15 kDa SEQ ID NOS:9 (nucleotide) and 74 (amino acid)	gb:NM_005101.1 /DEF=Homo sapiens interferon-stimulated protein, 15 kDa (ISG15), mRNA. /FEA=mRNA /GEN=ISG15 /PROD=interferon-stimulated protein, 15 kDa /DB_XREF=gi:4826773 /UG=Hs.833 interferon-stimulated protein, 15 kDa /FL=gb:M13755.1 gb:NM_005101.1	205483_s_at
Dopa decarboxylase (aromatic L-amino acid decarboxylase) SEQ ID NOS:10 (nucleotide) and 75 (amino acid)	gb:NM_000790.1 /DEF=Homo sapiens dopa decarboxylase (aromatic L-amino acid decarboxylase) (DDC), mRNA. /FEA=mRNA /GEN=DDC /PROD=dopa decarboxylase (aromatic L-amino aciddecarboxylase) /DB_XREF=gi:4503280 /UG=Hs.150403 dopa decarboxylase (aromatic L-amino acid decarboxylase) /FL=gb:BC000485.1 gb:M76180.1 gb:M88700.1 gb:NM_000790.1	205311_at

Serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 SEQ ID NOS:11 (nucleotide) and 76 (amino acid)	gb:NM_000602.1 /DEF=Homo sapiens serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (SERPINE1), mRNA. /FEA=mRNA /GEN=SERPINE1 /PROD=serine (or cysteine) proteinase inhibitor, cladeE (nexin, plasminogen activator inhibitor type 1), member1 /DB_XREF=gi:10835158 /UG=Hs.82085 serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 /FL=gb:NM_000602.1 gb:M16006.1	202628_s_at
FXYD domain-containing ion transport regulator 3 SEQ ID NOS:12 (nucleotide) and 77 (amino acid)	gb:BC005238.1 /DEF=Homo sapiens, FXYD domain-containing ion transport regulator 3, clone MGC:12265, mRNA, complete cds. /FEA=mRNA /PROD=FXYD domain-containing ion transport regulator3 /DB_XREF=gi:13528881 /UG=Hs.301350 FXYD domain-containing ion transport regulator 3 /FL=gb:NM_005971.2 gb:BC005238.1	202489_s_at
Putative integral membrane transporter SEQ ID NOS:13 (nucleotide) and 78 (amino acid)	gb:NM_018407.1 /DEF=Homo sapiens putative integral membrane transporter (LC27), mRNA. /FEA=mRNA /GEN=LC27 /PROD=putative integral membrane transporter /DB_XREF=gi:8923827 /FL=gb:NM_018407.1	208029_s_at
Protease inhibitor 3, skin-derived (SKALP) SEQ ID NOS:14 (nucleotide) and 79 (amino acid)	gb:NM_002638.1 /DEF=Homo sapiens protease inhibitor 3, skin-derived (SKALP) (PI3), mRNA. /FEA=mRNA /GEN=PI3 /PROD=protease inhibitor 3, skin-derived (SKALP) /DB_XREF=gi:4505786 /UG=Hs.112341 protease inhibitor 3, skin-derived (SKALP) /FL=gb:NM_002638.1	203691_at
Caudal type homeo box transcription factor 2 SEQ ID NOS:15 (nucleotide) and 80 (amino acid)	gb:U51096.1 /DEF=Human homeobox protein Cdx2 mRNA, complete cds. /FEA=mRNA /PROD=homeobox protein Cdx2 /DB_XREF=gi:1777773 /UG=Hs.77399 caudal type homeo box transcription factor 2 /FL=gb:U51096.1 gb:NM_001265.1	206387_at
Fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)	gb:NM_000142.2 /DEF=Homo sapiens fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism) (FGFR3), transcript variant 1, mRNA.	204379_s_at

SEQ ID NOS:16 (nucleotide) and 81 (amino acid)	/FEA=mRNA /GEN=FGFR3 /PROD=fibroblast growth factor receptor 3, isoform 1precursor /DB_XREF=gi:13112046 /UG=Hs.1420 fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism) /FL=gb:NM_000142.2 gb:M58051.1	
Hypothetical protein PP1665 SEQ ID NOS:17 (nucleotide), 18 (nucleotide) and 82 (amino acid)	Consensus includes gb:AL041124 /FEA=EST /DB_XREF=gi:5410060 /DB_XREF=est:DKFZp434D0316_s1 /CLONE=DKFZp434D0316 /UG=Hs.6748 hypothetical protein PP1665	213343_s_at
Protease inhibitor 3, skin-derived (SKALP) SEQ ID NOS:19 (nucleotide) and 83 (amino acid)	Cluster Incl. L10343:Huma elafin gene, complete cds /cds=(516,869) /gb=L10343 /gi=190337 /ug=Hs.112341 /len=871	41469_at
A kinase (PRKA) anchor protein (gravin) 12 SEQ ID NOS:20 (nucleotide) and 84 (amino acid)	gb:AB003476.1 /DEF=Homo sapiens mRNA for gravin, complete cds. /FEA=mRNA /PROD=gravin /DB_XREF=gi:2081606 /UG=Hs.788 A kinase (PRKA) anchor protein (gravin) 12 /FL=gb:AB003476.1	210517_s_at
Lymphocyte antigen 75 SEQ ID NOS:21 (nucleotide) and 85 (amino acid)	gb:NM_002349.1 /DEF=Homo sapiens lymphocyte antigen 75 (LY75), mRNA. /FEA=mRNA /GEN=LY75 /PROD=lymphocyte antigen 75 /DB_XREF=gi:4505052 /UG=Hs.153563 lymphocyte antigen 75 /FL=gb:AF011333.1 gb:AF064827.1 gb:NM_002349.1	205668_at
Mucin 5, subtypes A and C, tracheobronchial/gastric SEQ ID NOS:22 (nucleotide)	Consensus includes gb:AI521646 /FEA=EST /DB_XREF=gi:4435781 /DB_XREF=est:to66a06.x1 /CLONE=IMAGE:2183218 /UG=Hs.102482 mucin 5, subtype B, tracheobronchial	214385_s_at
Metallothionein 1G SEQ ID NOS:23 (nucleotide) and 86 (amino acid)	gb:NM_005950.1 /DEF=Homo sapiens metallothionein 1G (MT1G), mRNA. /FEA=mRNA /GEN=MT1G /PROD=metallothionein 1G /DB_XREF=gi:10835229 /UG=Hs.173451 metallothionein 1G /FL=gb:NM_005950.1	204745_x_at
Tumor necrosis factor	gb:NM_003823.1 /DEF=Homo sapiens	206467_x_at

receptor superfamily, member 6b, decoy SEQ ID NOS:24 (nucleotide) and 87 (amino acid)	tumor necrosis factor receptor superfamily, member 6b, decoy (TNFRSF6B), mRNA. /FEA=mRNA /GEN=TNFRSF6B /PROD=decoy receptor 3 /DB_XREF=gi:4507584 /UG=Hs.278556 tumor necrosis factor receptor superfamily, member 6b, decoy /FL=gb:AF104419.1 gb:NM_003823.1 gb:AF134240.1 gb:AF217794.1	
Mucin 3B SEQ ID NOS:25 (nucleotide) and 88 (amino acid)	Consensus includes gb:AB038783.1 /DEF=Homo sapiens MUC3B mRNA for intestinal mucin, partial cds. /FEA=mRNA /GEN=MUC3B /PROD=intestinal mucin /DB_XREF=gi:9929917 /UG=Hs.129782 mucin 3A, intestinal	214898_x_at
Metallothionein 1X SEQ ID NOS:26 (nucleotide) and 89 (amino acid)	gb:NM_005952.1 /DEF=Homo sapiens metallothionein 1X (MT1X), mRNA. /FEA=CDS /GEN=MT1X /PROD=metallothionein 1X /DB_XREF=gi:10835231 /UG=Hs.278462 metallothionein 1X /FL=gb:NM_005952.1	208581_x_at
GRO3 oncogene SEQ ID NOS:27 (nucleotide) and 90 (amino acid)	gb:NM_002090.1 /DEF=Homo sapiens GRO3 oncogene (GRO3), mRNA. /FEA=mRNA /GEN=GRO3 /PROD=GRO3 oncogene /DB_XREF=gi:4504156 /UG=Hs.89690 GRO3 oncogene /FL=gb:M36821.1 gb:NM_002090.1	207850_at
Transforming growth factor, beta-induced, 68kD SEQ ID NOS:28 (nucleotide) and 91 (amino acid)	gb:NM_000358.1 /DEF=Homo sapiens transforming growth factor, beta-induced, 68kD (TGFBI), mRNA. /FEA=mRNA /GEN=TGFBI /PROD=transforming growth factor, beta-induced, 68kD /DB_XREF=gi:4507466 /UG=Hs.118787 transforming growth factor, beta-induced, 68kD /FL=gb:BC000097.1 gb:BC004972.1 gb:M77349.1 gb:NM_000358.1	201506_at
Bone morphogenetic protein 7 (osteogenic protein 1) SEQ ID NOS:29 (nucleotide) and 92 (amino acid)	gb:M60316.1 /DEF=Human transforming growth factor-beta (tgf-beta) mRNA, complete cds. /FEA=mRNA /GEN=tgf-beta /PROD=transforming growth factor-beta /DB_XREF=gi:339563 /UG=Hs.170195 bone morphogenetic protein 7 (osteogenic protein 1) /FL=gb:M60316.1 gb:NM_001719.1	209591_s_at
Annexin A10 SEQ ID NOS:30	gb:AF196478.1 /DEF=Homo sapiens annexin 14 (ANX14) mRNA, complete cds. /FEA=mRNA /GEN=ANX14	210143_at

(nucleotide) and 93 (amino acid)	/PROD=annexin 14 /DB_XREF=gi:6274496 /UG=Hs.188401 annexin A10 /FL=gb:AF196478.1 gb:NM_007193.2	
Metallothionein 1F (functional) SEQ ID NOS:31 (nucleotide) and 94 (amino acid)	Consensus includes gb:M10943 /DEF=Human metallothionein-If gene (hMT-If) /FEA=CDS /DB_XREF=gi:187540 /UG=Hs.203936 metallothionein 1F (functional)	217165_x_at
Annexin A1 SEQ ID NOS:32 (nucleotide) and 95 (amino acid)	gb:NM_000700.1 /DEF=Homo sapiens annexin A1 (ANXA1), mRNA. /FEA=mRNA /GEN=ANXA1 /PROD=annexin I /DB_XREF=gi:4502100 /UG=Hs.78225 annexin A1 /FL=gb:BC001275.1 gb:NM_000700.1	201012_at
Secretory leukocyte protease inhibitor (antileukoproteinase) SEQ ID NOS:33 (nucleotide) and 96 (amino acid)	gb:NM_003064.1 /DEF=Homo sapiens secretory leukocyte protease inhibitor (antileukoproteinase) (SLPI), mRNA. /FEA=mRNA /GEN=SLPI /PROD=secretory leukocyte protease inhibitor(antileukoproteinase) /DB_XREF=gi:4507064 /UG=Hs.251754 secretory leukocyte protease inhibitor (antileukoproteinase) /FL=gb:NM_003066.1 gb:AF114471.1 gb:NM_003064.1	203021_at
Polymeric immunoglobulin receptor SEQ ID NOS:34 (nucleotide) and 97 (amino acid)	gb:NM_002644.1 /DEF=Homo sapiens polymeric immunoglobulin receptor (PIGR), mRNA. /FEA=mRNA /GEN=PIGR /PROD=polymeric immunoglobulin receptor /DB_XREF=gi:11342673 /UG=Hs.288579 polymeric immunoglobulin receptor /FL=gb:NM_002644.1	204213_at
Carcinoembryonic antigen-related cell adhesion molecule 5 SEQ ID NOS:35 (nucleotide) and 98 (amino acid)	gb:NM_004363.1 /DEF=Homo sapiens carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5), mRNA. /FEA=mRNA /GEN=CEACAM5 /PROD=carcinoembryonic antigen-related cell adhesionmolecule 5 /DB_XREF=gi:11386170 /UG=Hs.220529 carcinoembryonic antigen-related cell adhesion molecule 5 /FL=gb:NM_004363.1 gb:M29540.1	201884_at
Protein tyrosine phosphatase, receptor type, N polypeptide 2	gb:NM_002847.1 /DEF=Homo sapiens protein tyrosine phosphatase, receptor type, N polypeptide 2 (PTPRN2), mRNA.	203029_s_at

SEQ ID NOS:36 (nucleotide) and 99 (amino acid)	/FEA=mRNA /GEN=PTPRN2 /PROD=protein tyrosine phosphatase, receptor type, Npolypeptide 2 /DB_XREF=gi:11386148 /UG=Hs.74624 protein tyrosine phosphatase, receptor type, N polypeptide 2 /FL=gb:NM_002847.1 gb:U66702.1 gb:AF007555.1	
Cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7) SEQ ID NOS:37 (nucleotide) and 100 (amino acid)	gb:NM_000492.2 /DEF=Homo sapiens cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7) (CFTR), mRNA. /FEA=mRNA /GEN=CFTR /PROD=cystic fibrosis transmembrane conductanceregulator, ATP-binding cassette (sub-family C, member 7) /DB_XREF=gi:6995995 /UG=Hs.663 cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7) /FL=gb:NM_000492.2	205043_at
DVS27-related protein SEQ ID NOS:38 (nucleotide) and 101 (amino acid)	gb:AB024518.1 /DEF=Homo sapiens mRNA for DVS27-related protein, complete cds. /FEA=mRNA /GEN=DVS27 /PROD=DVS27-related protein /DB_XREF=gi:4520327 /UG=Hs.58589 glycogenin 2 /FL=gb:AB024518.1	209821_at
Insulin-like growth factor binding protein 2 (36kD) SEQ ID NOS:39 (nucleotide) and 102 (amino acid)	gb:NM_000597.1 /DEF=Homo sapiens insulin-like growth factor binding protein 2 (36kD) (IGFBP2), mRNA. /FEA=mRNA /GEN=IGFBP2 /PROD=insulin-like growth factor binding protein 2(36kD) /DB_XREF=gi:10835156 /UG=Hs.162 insulin-like growth factor binding protein 2 (36kD) /FL=gb:NM_000597.1 gb:BC004312.1 gb:M35410.1	202718_at
Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein SEQ ID NOS:40 (nucleotide) and 103 (amino acid)	gb:NM_002167.1 /DEF=Homo sapiens inhibitor of DNA binding 3, dominant negative helix-loop-helix protein (ID3), mRNA. /FEA=mRNA /GEN=ID3 /PROD=inhibitor of DNA binding 3, dominant negativehelix-loop-helix protein /DB_XREF=gi:10835060 /UG=Hs.76884 inhibitor of DNA binding 3, dominant negative helix-loop-helix protein /FL=gb:NM_002167.1	207826_s_at
Phospholipase A2, group IIA (platelets,	Consensus includes gb:X00452.1 /DEF=Human mRNA for DC classII	203649_s_at

synovial fluid) SEQ ID NOS:41 (nucleotide) and 104 (amino acid)	histocompatibility antigen alpha-chain. /FEA=mRNA /PROD=DC classII histocompatibility antigenalpha-chain /DB_XREF=gi:32265 /UG=Hs.198253 major histocompatibility complex, class II, DQ alpha 1	
Purkinje cell protein 4 SEQ ID NOS:42 (nucleotide) and 105 (amino acid)	gb:NM_006198.1 /DEF=Homo sapiens Purkinje cell protein 4 (PCP4), mRNA. /FEA=mRNA /GEN=PCP4 /PROD=Purkinje cell protein 4 /DB_XREF=gi:5453857 /UG=Hs.80296 Purkinje cell protein 4 /FL=gb:U52969.1 gb:NM_006198.1	205549_at
G protein-coupled receptor 49 SEQ ID NOS:43 (nucleotide), 44 (nucleotide) and 106 (amino acid)	Consensus includes gb:AL524520 /FEA=EST /DB_XREF=gi:12788013 /DB_XREF=est:AL524520 /CLONE=CS0DC007YG21 (3 prime) /UG=Hs.285529 G protein-coupled receptor 49	213880_at
Fucosyltransferase 3 (galactoside 3(4)-L-fucosyltransferase, Lewis blood group included) SEQ ID NOS:45 (nucleotide), 46 (nucleotide) and 107 (amino acid)	Consensus includes gb:AW080549 /FEA=EST /DB_XREF=gi:6035701 /DB_XREF=est:xc33a08.x1 /CLONE=IMAGE:2586038 /UG=Hs.169238 fucosyltransferase 3 (galactoside 3(4)-L-fucosyltransferase, Lewis blood group included)	214088_s_at
Interferon, alpha-inducible protein 27 SEQ ID NOS:47 (nucleotide) and 108 (amino acid)	gb:NM_005532.1 /DEF=Homo sapiens interferon, alpha-inducible protein 27 (IFI27), mRNA. /FEA=mRNA /GEN=IFI27 /PROD=interferon, alpha-inducible protein 27 /DB_XREF=gi:5031780 /UG=Hs.278613 interferon, alpha-inducible protein 27 /FL=gb:NM_005532.1	202411_at
Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 SEQ ID NOS:48 (nucleotide) and 109 (amino acid)	gb:NM_002639.1 /DEF=Homo sapiens serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 (SERPINB5), mRNA. /FEA=mRNA /GEN=SERPINB5 /PROD=serine (or cysteine) proteinase inhibitor, cladeB (ovalbumin), member 5 /DB_XREF=gi:4505788 /UG=Hs.55279 serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5	204855_at

	/FL=gb:NM_002639.1 gb:U04313.1	
Homo sapiens CD44 isoform RC (CD44) mRNA, complete cds SEQ ID NOS:49 (nucleotide) and 110 (amino acid)	gb:AF098641.1 /DEF=Homo sapiens CD44 isoform RC (CD44) mRNA, complete cds. /FEA=mRNA /GEN=CD44 /PROD=CD44 isoform RC /DB_XREF=gi:3832517 /UG=Hs.306278 Homo sapiens CD44 isoform RC (CD44) mRNA, complete cds /FL=gb:AF098641.1	210916_s_at
Solute carrier family 7 (cationic amino acid transporter, y+ system), member 8 SEQ ID NOS:50 (nucleotide) and 111 (amino acid)	gb:NM_012244.1 /DEF=Homo sapiens solute carrier family 7 (cationic amino acid transporter, y+ system), member 8 (SLC7A8), mRNA. /FEA=mRNA /GEN=SLC7A8 /PROD=solute carrier family 7 (cationic amino acidtransporter, y+ system), member 8 /DB_XREF=gi:6912669 /UG=Hs.22891 solute carrier family 7 (cationic amino acid transporter, y+ system), member 8 /FL=gb:AB037669.1 gb:AF171669.1 gb:NM_012244.1	202752_x_at
Membrane protein, palmitoylated 1 (55kD) SEQ ID NOS:51 (nucleotide) and 112 (amino acid)	gb:NM_002436.2 /DEF=Homo sapiens membrane protein, palmitoylated 1 (55kD) (MPP1), mRNA. /FEA=mRNA /GEN=MPP1 /PROD=palmitoylated membrane protein 1 /DB_XREF=gi:6006024 /UG=Hs.1861 membrane protein, palmitoylated 1 (55kD) /FL=gb:BC002392.1 gb:M64925.1 gb:NM_002436.2	202974_at
Tumor protein p53 (Li-Fraumeni syndrome) SEQ ID NOS:52 (nucleotide) and 113 (amino acid)	gb:K03199.1 /DEF=Human p53 cellular tumor antigen mRNA, complete cds. /FEA=mRNA /GEN=TP53 /DB_XREF=gi:189478 /UG=Hs.1846 tumor protein p53 (Li-Fraumeni syndrome) /FL=gb:K03199.1	211300_s_at
S100 calcium-binding protein P SEQ ID NOS:53 (nucleotide) and 114 (amino acid)	gb:NM_005980.1 /DEF=Homo sapiens S100 calcium-binding protein P (S100P), mRNA. /FEA=mRNA /GEN=S100P /PROD=S100 calcium-binding protein P /DB_XREF=gi:5174662 /UG=Hs.2962 S100 calcium-binding protein P /FL=gb:NM_005980.1	204351_at
Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	gb:AF119873.1 /DEF=Homo sapiens PRO2275 mRNA, complete cds. /FEA=mRNA /PROD=PRO2275 /DB_XREF=gi:7770182 /UG=Hs.297681 serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase,	211429_s_at

SEQ ID NOS:54 (nucleotide) and 115 (amino acid)	antitrypsin), member 1 /FL=gb:AF119873.1	
Eukaryotic translation initiation factor 5A SEQ ID NOS:55 (nucleotide) and 116 (amino acid)	gb:NM_001970.1 /DEF=Homo sapiens eukaryotic translation initiation factor 5A (EIF5A), mRNA. /FEA=mRNA /GEN=EIF5A /PROD=eukaryotic translation initiation factor 5A /DB_XREF=gi:4503544 /UG=Hs.119140 eukaryotic translation initiation factor 5A /FL=gb:BC000751.1 gb:BC001832.1 gb:M23419.1 gb:NM_001970.1	201123_s_at
Old astrocyte specifically induced substance SEQ ID NOS:56 (nucleotide), 57 (nucleotide) and 117 (amino acid)	Consensus includes gb:AF055009.1 /DEF=Homo sapiens clone 24747 mRNA sequence. /FEA=mRNA /DB_XREF=gi:3005731 /UG=Hs.13456 Homo sapiens clone 24747 mRNA sequence	213059_at
UDP glycosyltransferase 1 family, polypeptide A3 SEQ ID NOS:58 (nucleotide) and 118 (amino acid)	gb:NM_019093.1 /DEF=Homo sapiens UDP glycosyltransferase 1 family, polypeptide A3 (UGT1A3), mRNA. /FEA=CDS /GEN=UGT1A3 /PROD=UDP glycosyltransferase 1 family, polypeptideA3 /DB_XREF=gi:13487899 /UG=Hs.326543 UDP glycosyltransferase 1 family, polypeptide A3 /FL=gb:NM_019093.1	208596_s_at
Alpha-2-HS-glycoprotein SEQ ID NOS:59 (nucleotide) and 119 (amino acid)	gb:AF130057.1 /DEF=Homo sapiens clone FLB5539 PRO1454 mRNA, complete cds. /FEA=mRNA /PROD=PRO1454 /DB_XREF=gi:11493420 /UG=Hs.323288 Homo sapiens clone FLB5539 PRO1454 mRNA, complete cds /FL=gb:AF130057.1	210929_s_at
ESTs, Highly similar to A39092 glucuronosyltransferase [H.sapiens] SEQ ID NOS:60 (nucleotide), 61 (nucleotide) and 120 (amino acid)	Consensus includes gb:AV691323 /FEA=EST /DB_XREF=gi:10293186 /DB_XREF=est:AV691323 /CLONE=GKCEWF11 /UG=Hs.2056 UDP glycosyltransferase 1 family, polypeptide A9	215125_s_at
UDP glycosyltransferase 1 family, polypeptide A1	gb:NM_000463.1 /DEF=Homo sapiens UDP glycosyltransferase 1 family, polypeptide A1 (UGT1A1), mRNA. /FEA=mRNA /GEN=UGT1A1	207126_x_at

SEQ ID NOS:62 (nucleotide) and 121 (amino acid)	/PROD=UDP glycosyltransferase 1 family, polypeptideA1 /DB_XREF=gi:8850235 /UG=Hs.278896 UDP glycosyltransferase 1 family, polypeptide A1 /FL=gb:M57899.1 gb:NM_000463.1	
Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 SEQ ID NOS:63 (nucleotide) and 122 (amino acid)	gb:NM_000295.1 /DEF=Homo sapiens serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 (SERPINA1), mRNA. /FEA=mRNA /GEN=SERPINA1 /PROD=serine (or cysteine) proteinase inhibitor, cladeA (alpha-1 antiproteinase, antitrypsin), member 1 /DB_XREF=gi:4505792 /UG=Hs.297681 serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 /FL=gb:AF130068.1 gb:M11465.1 gb:K01396.1 gb:NM_000295.1	202833_s_at
Nerve growth factor receptor (TNFRSF16) associated protein 1 SEQ ID NOS:64 (nucleotide) and 123 (amino acid)	gb:NM_014380.1 /DEF=Homo sapiens p75NTR-associated cell death executor; ovarian granulosa cell protein (13kD) (DXS6984E), mRNA. /FEA=mRNA /GEN=DXS6984E /PROD=p75NTR-associated cell death executor; ovariangranulosa cell protein (13kD) /DB_XREF=gi:7657043 /UG=Hs.17775 p75NTR-associated cell death executor; ovarian granulosa cell protein (13kD) /FL=gb:NM_014380.1 gb:AF187064.1	217963_s_at
Collagen, type XVIII, alpha 1 SEQ ID NOS:65 (nucleotide) and 124 (amino acid)	Consensus includes gb:NM_030582.1 /DEF=Homo sapiens collagen, type XVIII, alpha 1 (COL18A1), mRNA. /FEA=CDS /GEN=COL18A1 /PROD=collagen, type XVIII, alpha 1 /DB_XREF=gi:13385619 /UG=Hs.78409 collagen, type XVIII, alpha 1 /FL=gb:NM_030582.1 gb:AF018081.1 gb:AF184060.1 gb:NM_016214.1	209081_s_at
Collagen, type IX, alpha 3 SEQ ID NOS:66 (nucleotide) and 125 (amino acid)	gb:NM_001853.1 /DEF=Homo sapiens collagen, type IX, alpha 3 (COL9A3), mRNA. /FEA=mRNA /GEN=COL9A3 /PROD=collagen, type IX, alpha 3 /DB_XREF=gi:4502966 /UG=Hs.53563 collagen, type IX, alpha 3 /FL=gb:L41162.1 gb:NM_001853.1	204724_s_at

The biomarkers have expression levels in the cells that are dependent on the activity of the EGFR signal transduction pathway and that are also highly correlated with EGFR modulator sensitivity exhibited by the cells. Biomarkers serve as useful molecular tools for predicting a response to EGFR modulators, preferably biological molecules, small molecules, and the like that affect EGFR kinase activity via direct or indirect inhibition or antagonism of EGFR kinase function or activity.

EGFR MODULATORS

As used herein, the term "EGFR modulator" is intended to mean a compound or drug that is a biological molecule or a small molecule that directly or indirectly modulates EGFR activity or the EGFR signal transduction pathway. Thus, compounds or drugs as used herein is intended to include both small molecules and biological molecules. Direct or indirect modulation includes activation or inhibition of EGFR activity or the EGFR signal transduction pathway. In one aspect, inhibition refers to inhibition of the binding of EGFR to an EGFR ligand such as, for example, EGF. In another aspect, inhibition refers to inhibition of the kinase activity of EGFR.

EGFR modulators include, for example, EGFR specific ligands, small molecule EGFR inhibitors, and EGFR monoclonal antibodies. In one aspect, the EGFR modulator inhibits EGFR activity and/or inhibits the EGFR signal transduction pathway. In another aspect, the EGFR modulator is an EGFR monoclonal antibody that inhibits EGFR activity and/or inhibits the EGFR signal transduction pathway.

EGFR modulators include biological molecules or small molecules. Biological molecules include all lipids and polymers of monosaccharides, amino acids, and nucleotides having a molecular weight greater than 450. Thus, biological molecules include, for example, oligosaccharides and polysaccharides; oligopeptides, polypeptides, peptides, and proteins; and oligonucleotides and polynucleotides. Oligonucleotides and polynucleotides include, for example, DNA and RNA.

Biological molecules further include derivatives of any of the molecules described above. For example, derivatives of biological molecules include lipid and glycosylation derivatives of oligopeptides, polypeptides, peptides, and proteins.

Derivatives of biological molecules further include lipid derivatives of oligosaccharides and polysaccharides, e.g., lipopolysaccharides. Most typically,

biological molecules are antibodies, or functional equivalents of antibodies. Functional equivalents of antibodies have binding characteristics comparable to those of antibodies, and inhibit the growth of cells that express EGFR. Such functional equivalents include, for example, chimerized, humanized, and single chain antibodies as well as fragments thereof.

Functional equivalents of antibodies also include polypeptides with amino acid sequences substantially the same as the amino acid sequence of the variable or hypervariable regions of the antibodies. An amino acid sequence that is substantially the same as another sequence, but that differs from the other sequence by means of one or more substitutions, additions, and/or deletions, is considered to be an equivalent sequence. Preferably, less than 50%, more preferably less than 25%, and still more preferably less than 10%, of the number of amino acid residues in a sequence are substituted for, added to, or deleted from the protein.

The functional equivalent of an antibody is preferably a chimerized or humanized antibody. A chimerized antibody comprises the variable region of a non-human antibody and the constant region of a human antibody. A humanized antibody comprises the hypervariable region (CDRs) of a non-human antibody. The variable region other than the hypervariable region, e.g., the framework variable region, and the constant region of a humanized antibody are those of a human antibody.

Suitable variable and hypervariable regions of non-human antibodies may be derived from antibodies produced by any non-human mammal in which monoclonal antibodies are made. Suitable examples of mammals other than humans include, for example, rabbits, rats, mice, horses, goats, or primates.

Functional equivalents further include fragments of antibodies that have binding characteristics that are the same as, or are comparable to, those of the whole antibody. Suitable fragments of the antibody include any fragment that comprises a sufficient portion of the hypervariable (i.e., complementarity determining) region to bind specifically, and with sufficient affinity, to EGFR tyrosine kinase to inhibit growth of cells that express such receptors.

Such fragments may, for example, contain one or both Fab fragments or the $F(ab')_2$ fragment. Preferably, the antibody fragments contain all six complementarity

determining regions of the whole antibody, although functional fragments containing fewer than all of such regions, such as three, four, or five CDRs, are also included.

In one aspect, the fragments are single chain antibodies, or Fv fragments. Single chain antibodies are polypeptides that comprise at least the variable region of the heavy chain of the antibody linked to the variable region of the light chain, with or without an interconnecting linker. Thus, Fv fragment comprises the entire antibody combining site. These chains may be produced in bacteria or in eukaryotic cells.

The antibodies and functional equivalents may be members of any class of immunoglobulins, such as IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof. In one aspect, the antibodies are members of the IgG1 subclass. The functional equivalents may also be equivalents of combinations of any of the above classes and subclasses.

In one aspect, EGFR antibodies can be selected from chimerized, humanized, fully human, and single chain antibodies derived from the murine antibody 225 described in U.S. Patent No. 4,943,533 to Mendelsohn et al., including, for example, cetuximab.

In another aspect, the EGFR antibody can be selected from the antibodies described in U.S. Patent No. 6,235,883 to Jakobovits et al., U.S. Patent No. 5,558,864 to Bendi et al., and U.S. Patent No. 5,891,996 to Mateo de Acosta del Rio et al.

In addition to the biological molecules discussed above, the EGFR modulators useful in the invention may also be small molecules. Any molecule that is not a biological molecule is considered herein to be a small molecule. Some examples of small molecules include organic compounds, organometallic compounds, salts of organic and organometallic compounds, saccharides, amino acids, and nucleotides. Small molecules further include molecules that would otherwise be considered biological molecules, except their molecular weight is not greater than 450. Thus, small molecules may be lipids, oligosaccharides, oligopeptides, and oligonucleotides and their derivatives, having a molecular weight of 450 or less.

It is emphasized that small molecules can have any molecular weight. They are merely called small molecules because they typically have molecular weights less than 450. Small molecules include compounds that are found in nature as well as synthetic compounds. In one embodiment, the EGFR modulator is a small molecule

that inhibits the growth of tumor cells that express EGFR. In another embodiment, the EGFR modulator is a small molecule that inhibits the growth of refractory tumor cells that express EGFR. In yet another embodiment, the EGFR modulator is erlotinib HCl or gefitinib.

Numerous small molecules have been described as being useful to inhibit EGFR. For example, U.S. Patent No. 5,656,655 to Spada et al. discloses styryl substituted heteroaryl compounds that inhibit EGFR. The heteroaryl group is a monocyclic ring with one or two heteroatoms, or a bicyclic ring with 1 to about 4 heteroatoms, the compound being optionally substituted or polysubstituted.

U.S. Patent No. 5,646,153 to Spada et al. discloses bis mono and/or bicyclic aryl heteroaryl, carbocyclic, and heterocarbocyclic compounds that inhibit EGFR.

U.S. Patent No. 5,679,683 to Bridges et al. discloses tricyclic pyrimidine compounds that inhibit the EGFR. The compounds are fused heterocyclic pyrimidine derivatives described at column 3, line 35 to column 5, line 6.

U.S. Patent No. 5,616,582 to Barker discloses quinazoline derivatives that have receptor tyrosine kinase inhibitory activity.

Fry et al., Science 265, 1093-1095 (1994) in Figure 1 discloses a compound having a structure that inhibits EGFR.

Osherov et al. disclose tyrphostins that inhibit EGFR/HER1 and HER 2, particularly those in Tables I, II, III, and IV.

U.S. Patent No. 5,196,446 to Levitzki et al. discloses heteroarylethenediyl or heteroarylethendeiylaryl compounds that inhibit EGFR; particularly from column 2, line 42 to column 3, line 40.

Panek et al., Journal of Pharmacology and Experimental Therapeutics 283, 1433-1444 (1997) discloses a compound identified as PD166285 that inhibits the EGFR, PDGFR, and FGFR families of receptors. PD166285 is identified as 6-(2,6-dichlorophenyl)-2-(4-(2-diethylaminoethyoxy)phenylamino)-8-methyl-8H-pyrido(2,3-d)pyrimidin-7-one having the structure shown in Figure 1 on page 1436.

BIOMARKERS AND BIOMARKER SETS

The invention includes individdual biomarkers and biomarker sets having both diagnostic and prognostic value in disease areas in which signaling through EGFR or

the EGFR pathway is of importance, e.g., in cancers or tumors, in immunological disorders, conditions or dysfunction, or in disease states in which cell signaling and/or cellular proliferation controls are abnormal or aberrant. The biomarker sets comprise a plurality of biomarkers such as, for example, a plurality of the biomarkers provided in Table 1, that highly correlate with resistance or sensitivity to one or more EGFR modulators.

The biomarker sets of the invention enable one to predict or reasonably foretell the likely effect of one or more EGFR modulators in different biological systems or for cellular responses. The biomarker sets can be used in *in vitro* assays of EGFR modulator response by test cells to predict *in vivo* outcome. In accordance with the invention, the various biomarker sets described herein, or the combination of these biomarker sets with other biomarkers or markers, can be used, for example, to predict how patients with cancer might respond to therapeutic intervention with one or more EGFR modulators.

A biomarker set of cellular gene expression patterns correlating with sensitivity or resistance of cells following exposure of the cells to one or more EGFR modulators provides a useful tool for screening one or tumor samples before treatment with the EGFR modulator. The screening allows a prediction of cells of a tumor sample exposed to one or more EGFR modulators, based on the expression results of the biomarker set, as to whether or not the tumor, and hence a patient harboring the tumor, will or will not respond to treatment with the EGFR modulator.

The biomarker or biomarker set can also be used as described herein for monitoring the progress of disease treatment or therapy in those patients undergoing treatment for a disease involving an EGFR modulator.

The biomarkers also serve as targets for the development of therapies for disease treatment. Such targets may be particularly applicable to treatment of colon disease, such as colon cancers or tumors. Indeed, because these biomarkers are differentially expressed in sensitive and resistant cells, their expression patterns are correlated with relative intrinsic sensitivity of cells to treatment with EGFR modulators. Accordingly, the biomarkers highly expressed in resistant cells may serve as targets for the development of new therapies for the tumors which are resistant to EGFR modulators, particularly EGFR inhibitors.

MICROARRAYS

The invention also includes specialized microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, comprising one or more biomarkers, showing expression profiles that correlate with either sensitivity or resistance to one or more EGFR modulators. Such microarrays can be employed in *in vitro* assays for assessing the expression level of the biomarkers in the test cells from tumor biopsies, and determining whether these test cells are likely to be resistant or sensitive to EGFR modulators. For example, a specialized microarray can be prepared using all the biomarkers, or subsets thereof, as described herein and shown in Table 1. Cells from a tissue or organ biopsy can be isolated and exposed to one or more of the EGFR modulators. Following application of nucleic acids isolated from both untreated and treated cells to one or more of the specialized microarrays, the pattern of gene expression of the tested cells can be determined and compared with that of the biomarker pattern from the control panel of cells used to create the biomarker set on the microarray. Based upon the gene expression pattern results from the cells that underwent testing, it can be determined if the cells show a resistant or a sensitive profile of gene expression. Whether or not the tested cells from a tissue or organ biopsy will respond to one or more of the EGFR modulators and the course of treatment or therapy can then be determined or evaluated based on the information gleaned from the results of the specialized microarray analysis.

ANTIBODIES

The invention also includes antibodies, including polyclonal or monoclonal, directed against one or more of the polypeptide biomarkers. Such antibodies can be used in a variety of ways, for example, to purify, detect, and target the biomarkers of the invention, including both *in vitro* and *in vivo* diagnostic, detection; screening,. and/or therapeutic methods.

KITS

The invention also includes kits for determining or predicting whether a patient would be susceptible or resistant to a treatment that comprises one or more

EGFR modulators. The patient may have a cancer or tumor such as, for example, a colon cancer or tumor. Such kits would be useful in a clinical setting for use in testing a patient's biopsied tumor or cancer samples, for example, to determine or predict if the patient's tumor or cancer will be resistant or sensitive to a given treatment or therapy with an EGFR modulator. The kit comprises a suitable container that comprises: one or more microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, that comprise those biomarkers that correlate with resistance and sensitivity to EGFR modulators, particularly EGFR inhibitors; one or more EGFR modulators for use in testing cells from patient tissue specimens or patient samples; and instructions for use. In addition, kits contemplated by the invention can further include, for example, reagents or materials for monitoring the expression of biomarkers of the invention at the level of mRNA or protein, using other techniques and systems practiced in the art such as, for example, RT-PCR assays, which employ primers designed on the basis of one or more of the biomarkers described herein, immunoassays, such as enzyme linked immunosorbent assays (ELISAs), immunoblotting, e.g., Western blots, or *in situ* hybridization, and the like, as further described herein.

APPLICATION OF BIOMARKERS AND BIOMARKER SETS

The biomarkers and biomarker sets may be used in different applications. Biomarker sets can be built from any combination of biomarkers listed in Table 1 to make predictions about the likely effect of any EGFR modulator in different biological systems. The various biomarkers and biomarkers sets described herein can be used, for example, as diagnostic or prognostic indicators in disease management, to predict how patients with cancer might respond to therapeutic intervention with compounds that modulate the EGFR, and to predict how patients might respond to therapeutic intervention that modulates signaling through the entire EGFR regulatory pathway.

While the data described herein were generated in cell lines that are routinely used to screen and identify compounds that have potential utility for cancer therapy, the biomarkers have both diagnostic and prognostic value in other diseases areas in which signaling through EGFR or the EGFR pathway is of importance, e.g., in

immunology, or in cancers or tumors in which cell signaling and/or proliferation controls have gone awry.

In accordance with the invention, cells from a patient tissue sample, e.g., a tumor or cancer biopsy, can be assayed to determine the expression pattern of one or more biomarkers prior to treatment with one or more EGFR modulators. Success or failure of a treatment can be determined based on the biomarker expression pattern of the cells from the test tissue (test cells), e.g., tumor or cancer biopsy, as being relatively similar or different from the expression pattern of a control set of the one or more biomarkers. Thus, if the test cells show a biomarker expression profile which corresponds to that of the biomarkers in the control panel of cells which are sensitive to the EGFR modulator, it is highly likely or predicted that the individual's cancer or tumor will respond favorably to treatment with the EGFR modulator. By contrast, if the test cells show a biomarker expression pattern corresponding to that of the biomarkers of the control panel of cells which are resistant to the EGFR modulator, it is highly likely or predicted that the individual's cancer or tumor will not respond to treatment with the EGFR modulator.

The invention also provides a method of monitoring the treatment of a patient having a disease treatable by one or more EGFR modulators. The isolated test cells from the patient's tissue sample, e.g., a tumor biopsy or tumor sample, can be assayed to determine the expression pattern of one or more biomarkers before and after exposure to an EGFR modulator wherein, preferably, the EGFR modulator is an EGFR inhibitor. The resulting biomarker expression profile of the test cells before and after treatment is compared with that of one or more biomarkers as described and shown herein to be highly expressed in the control panel of cells that are either resistant or sensitive to an EGFR modulator. Thus, if a patient's response is sensitive to treatment by an EGFR modulator, based on correlation of the expression profile of the one or biomarkers, the patient's treatment prognosis can be qualified as favorable and treatment can continue. Also, if, after treatment with an EGFR modulator, the test cells don't show a change in the biomarker expression profile corresponding to the control panel of cells that are sensitive to the EGFR modulator, it can serve as an indicator that the current treatment should be modified, changed, or even discontinued. This monitoring process can indicate success or failure of a patient's

treatment with an EGFR modulator and such monitoring processes can be repeated as necessary or desired.

The biomarkers of the invention can be used to predict an outcome prior to having any knowledge about a biological system. Essentially, a biomarker can be considered to be a statistical tool. Biomarkers are useful primarily in predicting the phenotype that is used to classify the biological system. In an embodiment of the invention, the goal of the prediction is to classify cancer cells as having an active or inactive EGFR pathway. Cancer cells with an inactive EGFR pathway can be considered resistant to treatment with an EGFR modulator. An inactive EGFR pathway is defined herein as a non-significant expression of the EGFR or by a classification as "resistant" or "sensitive" based on the IC_{50} value of each colon cell line to EGFR inhibitor compound as exemplified herein.

However, although the complete function of all of the biomarkers are not currently known, some of the biomarkers are likely to be directly or indirectly involved in the EGFR signaling pathway. In addition, some of the biomarkers may function in the metabolic or other resistance pathways specific to the EGFR modulators tested. Notwithstanding, knowledge about the function of the biomarkers is not a requisite for determining the accuracy of a biomarker according to the practice of the invention.

EXAMPLES:

EXAMPLE 1 - Identification of Biomarkers

The biomarkers of Table 1 were identified as follows.

Colon Tumors and Patients:

Forty colon tumors collected from the University of London between 1998 and 2002. The median age of the patients was 70 years (range: 26-91 years). The patients were diagnosed as follows: 6 patients were designated as Duke's A, 14 as Duke's B, and 20 as Duke's C. None of the patients were treated pre-operatively, and 13 were treated post-operatively.

Determination of Relative Drug Sensitivity in Colon Cancer Cell Lines:

The cell line filtering process used is illustrated in FIG. 2.

The colon cancer cell lines were grown using standard cell culture conditions: RPMI 1640 supplemented to contain 10% fetal bovine serum, 100 IU/ml penicillin, 100 mg/ml streptomycin, 2 mM L-glutamine and 10 mM Hepes (all from GibcoBRL, Rockville, MD). Twenty-one colon cancer cell lines were examined for their relative sensitivity to a pair of small molecule EGFR inhibitors, erlotinib HCl and gefitinib. Cytotoxicity was assessed in cells by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2H-tetrazolium, inner salt)assay (T.L. Riss et al., 1992, Mol. Biol. Cell, 3 (Suppl.):184a). To carry out the assays, the colon cancer cells were plated at 4,000 cells/well in 96 well microtiter plates and 24 hours later serial diluted drugs were added. The concentration range for the EGFR inhibitor compounds used in the cytotoxicity assays was 50 ug/ml to 0.0016 ug/ml (roughly 100 uM to 0.0032 uM). The cells were incubated at 37 °C for 72 hours at which time the tetrazolium dye MTS (333 ug/ml final concentration in combination with the electron coupling agent phenazine methosulfate) was added. A dehydrogenase enzyme in live cells reduces the MTS to a form that absorbs light at 492 nm that can be quantified spectrophotometrically. The greater the absorbency, the greater the number of live cells. The results, provided below in Table 2 and FIG. 3, are expressed as an IC50, which is the drug concentration required to inhibit cell proliferation to 50% of that of untreated cells.

Table 2 - Colon Cell Lines

Cell Line	ATCC No.	Avg. IC50
CaCo2	HTB-37	5.4
Colo 201	CCL-224	10+
Colo 205	CCL-222	10+
CS-1		10+
Difi		1
DLD-1		20
Geo		3.6
HCT116	CCL-247	67+
HCT116S542		53

HCT-8	CCL-244	10+
HT-29	HTB-38	10+
Lovo	CCL-229LS174T	3
LS1034		68+
RKORM13		29
SW1116		20
SW403		6.2
SW480	CCL-228	10+
SW837	CCL-235	7
SW948		73+
T84	CCL-248	10+
WiDr		67+

Resistance/sensitivity classification:

Two separate analyses were performed using different cut-offs to define EGFR-inhibitor resistance. For the first (designated "6-15"), the 6 cell lines with an IC50 at or below 7 uM were defined as sensitive and the remaining 15 cell lines were defined as resistant. For the second (designated "3-18"), the 3 cell lines with an IC50 below 4 uM were defined as sensitive and the remaining 18 cell lines were defined as resistant.

Gene Expression Profiling:

RNA was isolated from 50-70% confluent cell lines or colon cancer tumor tissue using the Rneasy kits from Qiagen (Valencia, CA). The quality of RNA was checked by measuring the 28S:18: ribosomal RNA ratio using and Agilent 2100 bioanalyzer (Agilent Technologies, Rockville, MD). Concentration of total RNA was determined spectrophotemetrically. 10 ug of total RNA was used to prepare biotyinylated probes according to the Affymetrix Genechip Expression Analysis Technical Manual. Targets were hybridized to human HG-U133A gene chips according to the manufacturers instructions. Data were preprocessed using the MAS 5.0 software (Affymetrix, Santa Clara, CA). The trimmed mean intensity for each chip was scaled to 1,500 to account for minor differences in global chip intensity so

that the overall expression level for each sample is comparable.

Data Analysis

All 22,215 probes (gene sequences) present on the U133A chip were considered as potential predictive biomarkers. To restrict the analysis to gene sequences expressed at a moderate level in colon tumor(s), gene sequences without at least one expression value of 2X the mean value for the array (3000 expression units) were removed leaving 6988 gene sequences. Next, to identify genes with variable expression in colon tumors (and therefore more likely to be able to correlate with variability in response to treatment), gene sequences with a VARP value (using log10-transformed data) < 0.1 were removed leaving 745 gene sequences. Next, the same expression and variance filters were applied to the remaining 745 gene sequences using the colon cell line data, reducing to 332 gene sequences for analysis (FIG. 1).

The 332 gene sequences were then subjected to a two-sided T-test using the Resistance/sensitivity classifications of the cell lines described above (FIG. 3). A total of 12 gene sequences had a p-value of <0.05 for both analyses (T-test Results I, FIG. 4). For the "6-15" analysis, 19 gene sequences were found to have a p-value <0.05 (T-Test Results II, FIG. 5). For the "3-18" analysis, 29 gene sequences were found to have a p-value <0.05 (T-test Results III, FIG. 6). Table 1 provides the biomarkers identified using the two-sided T-test.

EXAMPLE 2 - Untreated Xenograph Profiles

In Example 1, biomarkers were identified using sensitivity resistance profiles of cell lines to gefitinib and erlotinib HCl. The present example provided efficacy data for cetuximab (C225) in the colon cancer xenograft models Geo (sensitive to C225) and HT29 (resistant to C225).

In Vivo Antitumor Testing

Tumors were propagated in nude mice as subcutaneous (sc) transplants using tumor fragments obtained from donor mice. Tumor passage occurred approximately every two to four weeks. Tumors were then allowed to grow to the pre-determined

size window (usually between 100-200 mg, tumors outside the range were excluded) and animals were evenly distributed to various treatment and control groups. Animals were treated with C225 (1 mg/mouse q3d X 10, 14, ip). Treated animals were checked daily for treatment related toxicity/mortality. Each group of animals was weighed before the initiation of treatment (Wt1) and then again following the last treatment dose (Wt2). The difference in body weight (Wt2-Wt1) provided a measure of treatment-related toxicity. Tumor response was determined by measurement of tumors with a caliper twice a week, until the tumors reached a predetermined target size of 1 gm or became necrotic. Tumor weights (mg) were estimated from the formula:

$$\text{Tumor weight} = (\text{length} \times \text{width}^2)/2$$

Antitumor activity was determined in terms of primary tumor growth inhibition. This was determined in two ways: (i) calculating the relative median tumor weight (MTW) of treated (T) and control (C) mice at various time points (effects were expressed as %T/C); and (ii) calculating the tumor growth delay (T-C value), defined as the difference in time (days) required for the treated tumors (T) to reach a predetermined target size compared to those of the control group (C). Statistical evaluations of data were performed using Gehan's generalized Wilcoxon test for comparisons of time to reach tumor target size (Gehan 1965). Statistical significance was declared at $p < 0.05$. Antitumor activity was defined as a continuous MTW $\%T/C \leq 50\%$ for at least 1 tumor volume doubling time (TVDT) any time after the start of treatment, where TVDT (tumor volume doubling time) = median time (days) for control tumors to reach target size – median time (days) for control tumors to reach half the target size. In addition, treatment groups had to be accompanied by a statistically significant tumor growth delay (T-C value) ($p < 0.05$) to be termed active.

Treated animals were checked daily for treatment related toxicity/mortality. When death occurred, the day of death was recorded. Treated mice dying prior to having their tumors reach target size were considered to have died from drug toxicity. No control mice died bearing tumors less than target size. Treatment groups with more than one death caused by drug toxicity were considered to have had excessively toxic treatments and their data were not included in the evaluation of the compound's antitumor efficacy.

Table 3 provides the resulting untreated xenograph profiles.

Table 3 - Untreated Xenograph Profiles

Biomarker	**Probe**	**Differential expression in Geo (sensitive) and HT-29 (resistant) untreated xenografts**	**Absence and Presence of HT-29 and Geo**
transforming growth factor, beta-induced, 68kD	201506_at	Higher 373X in Geo than HT-29 (Absent)	HT-29 Absent Geo Present
carcinoembryonic antigen-related cell adhesion molecule 5	201884_at	Higher 85X in Geo than HT-29 (Absent)	HT-29 Absent Geo Present
nerve growth factor receptor (TNFRSF16) associated protein 1	217963_s_at	Higher 50X in Geo than HT-29 (Absent)	HT-29 Absent Geo Present
carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)	211657_at	Higher 23X in Geo than HT-29(Absent)	HT-29 Absent Geo Present
annexin A1	201012_at	Higher 16X in Geo than HT-29 (Absent)	HT-29 Absent Geo Present
tumor protein p53 (Li-Fraumeni syndrome)	211300_s_at	Higher 11X in Geo than HT-29 (Absent)	HT-29 Absent Geo Present
DVS27-related protein	209821_at	Higher 9X in Geo than HT-29 (Absent)	HT-29 Absent Geo Present
cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)	205043_at	Higher 7X in Geo than HT-29 (Absent)	HT-29 Absent Geo Present
serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	211429_s_at	Higher 7X in Geo than HT-29 (Absent)	HT-29 Absent Geo Present
bone morphogenetic protein 7 (osteogenic protein 1)	209591_s_at	Higher 4X in Geo than HT-29 (Absent)	HT-29 Absent Geo Present
interferon-stimulated protein, 15 kDa	205483_s_at	Higher 3X in Geo than HT-29(Absent)	HT-29 Absent Geo Present
S100 calcium-binding protein P	204351_at	Higher 11X in Geo than HT-29	HT-29 Present Geo Present
carcinoembryonic	203757_s_at	Higher 8X in Geo than HT-	HT-29 Present

antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)		29	Geo Present
putative integral membrane transporter	208029_s_at	Higher 7X in Geo than HT-29	HT-29 Present Geo Present
cadherin 17, LI cadherin (liver-intestine)	209847_at	Higher 4X in Geo than HT-29	HT-29 Present Geo Present
FXYD domain-containing ion transport regulator 3	202489_s_at	Higher 3X in Geo than HT-29	HT-29 Present Geo Present
insulin-like growth factor binding protein 2 (36kD)	202718_at	Higher 3X in Geo than HT-29	HT-29 Present Geo Present
eukaryotic translation initiation factor 5A	201123_s_at	Higher 3X in Geo than HT-29	HT-29 Present Geo Present
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	204607_at	Higher 2X in Geo than HT-29	HT-29 Present Geo Present
serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	202833_s_at	Higher 21X in HT-29 than Geo	HT-29 Present Geo Present
transmembrane protease, serine 2	211689_s_at	Higher 7X in HT-29 than Geo	HT-29 Present Geo Present
protease inhibitor 3, skin-derived (SKALP)	41469_at	Higher 6X in HT-29 than Geo	HT-29 Present Geo Present
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5	204855_at	Higher 4X in HT-29 than Geo	HT-29 Present Geo Present
fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)	204379_s_at	Higher 3X in HT-29 than Geo	HT-29 Present Geo Present
mucin 3B	214898_x_at	Higher 3X in HT-29 than Geo	HT-29 Present Geo Present
fucosyltransferase 3 (galactoside 3(4)-L-fucosyltransferase, Lewis blood group included)	214088_s_at	Higher 3X in HT-29 than Geo	HT-29 Present Geo Present
phospholipase A2, group IIA (platelets,	203649_s_at	Higher 2X in HT-29 than Geo	HT-29 Present Geo Present

synovial fluid)			
A kinase (PRKA) anchor protein (gravin) 12	210517_s_at	Higher 339X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	202628_s_at	Higher 280X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
ESTs, Highly similar to A39092 glucuronosyltransferase [H.sapiens]	215125_s_at	Higher 75X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
Purkinje cell protein 4	205549_at	Higher 38X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
lectin, galactoside-binding, soluble, 1 (galectin 1)	201105_at	Higher 33X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
old astrocyte specifically induced substance	213059_at	Higher 29X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
UDP glycosyltransferase 1 family, polypeptide A3	208596_s_at	Higher 23X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
hypothetical protein PP1665	213343_s_at	Higher 21X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
membrane protein, palmitoylated 1 (55kD)	202974_at	Higher 9X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
caudal type homeo box transcription factor 2	206387_at	Higher 8X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
polymeric immunoglobulin receptor	204213_at	Higher 7X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
mucin 5, subtypes A and C, tracheobronchial/gastric	214385_s_at	Higher 6X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
metallothionein 1G	204745_x_at	Higher 2X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	207826_s_at	Higher 2X in HT-29 than Geo (Absent)	HT-29 Present Geo Absent
lymphocyte antigen 75	205668_at	not differentially expressed	HT-29 Present

			Geo Absent
secretory leukocyte protease inhibitor (antileukoproteinase)	203021_at	not differentially expressed	HT-29 Present Geo Absent
dopa decarboxylase (aromatic L-amino acid decarboxylase)	205311_at	not differentially expressed	HT-29 Present Geo Absent
G protein-coupled receptor 49	213880_at	not differentially expressed	HT-29 Present Geo Absent
interferon, alpha-inducible protein 27	202411_at	not differentially expressed	HT-29 Present Geo Absent
Homo sapiens CD44 isoform RC (CD44) mRNA, complete cds	210916_s_at	not differentially expressed	HT-29 Present Geo Absent
mucin 5, subtypes A and C, tracheobronchial/gastric	214303_x_at	absent in HT-29 and Geo	HT-29 Absent Geo Absent
UDP glycosyltransferase 1 family, polypeptide A1	207126_x_at	absent in HT-29 and Geo	HT-29 Absent Geo Absent
metallothionein 1F (functional)	217165_x_at	absent in HT-29 and Geo	HT-29 Absent Geo Absent
GRO3 oncogene	207850_at	absent in HT-29 and Geo	HT-29 Absent Geo Absent
protease inhibitor 3, skin-derived (SKALP)	203691_at	absent in HT-29 and Geo	HT-29 Absent Geo Absent
annexin A10	210143_at	absent in HT-29 and Geo	HT-29 Absent Geo Absent
protein tyrosine phosphatase, receptor type, N polypeptide 2	203029_s_at	absent in HT-29 and Geo	HT-29 Absent Geo Absent
solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	202752_x_at	absent in HT-29 and Geo	HT-29 Absent Geo Absent
collagen, type XVIII, alpha 1	209081_s_at	absent in HT-29 and Geo	HT-29 Absent Geo Absent
collagen, type IX, alpha 3	204724_s_at	absent in HT-29 and Geo	HT-29 Absent Geo Absent
alpha-2-HS-glycoprotein	210929_s_at	?	HT-29 Absent Geo Absent
metallothionein 1X	208581_x_at	?	HT-29 Absent Geo Absent
tumor necrosis factor receptor superfamily,	206467_x_at	?	HT-29 Absent Geo Absent

member 6b, decoy			

EXAMPLE 3 - PRODUCTION OF ANTIBODIES AGAINST THE BIOMARKERS

Antibodies against the biomarkers can be prepared by a variety of methods. For example, cells expressing an biomarker polypeptide can be administered to an animal to induce the production of sera containing polyclonal antibodies directed to the expressed polypeptides. In one aspect, the biomarker protein is prepared and isolated or otherwise purified to render it substantially free of natural contaminants, using techniques commonly practiced in the art. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity for the expressed and isolated polypeptide.

In one aspect, the antibodies of the invention are monoclonal antibodies (or protein binding fragments thereof). Cells expressing the biomarker polypeptide can be cultured in any suitable tissue culture medium, however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented to contain 10% fetal bovine serum (inactivated at about 56 °C), and supplemented to contain about 10 g/l nonessential amino acids, about 1,00 U/ml penicillin, and about 100 µg/ml streptomycin.

The splenocytes of immunized (and boosted) mice can be extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line can be employed in accordance with the invention, however, it is preferable to employ the parent myeloma cell line (SP2/0), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (1981, *Gastroenterology*, 80:225-232). The hybridoma cells obtained through such a selection are then assayed to identify those cell clones that secrete antibodies capable of binding to the polypeptide immunogen, or a portion thereof.

Alternatively, additional antibodies capable of binding to the biomarker polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens and, therefore, it is possible to obtain an antibody that binds to a second antibody. In accordance with this method, protein specific antibodies can be used to immunize an

animal, preferably a mouse. The splenocytes of such an immunized animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones that produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce the formation of further protein-specific antibodies.

EXAMPLE 4 - IMMUNOFLUORESCENCE ASSAYS

The following immunofluorescence protocol may be used, for example, to verify EGFR biomarker protein expression on cells or, for example, to check for the presence of one or more antibodies that bind EGFR biomarkers expressed on the surface of cells. Briefly, Lab-Tek II chamber slides are coated overnight at 4 °C with 10 micrograms/milliliter (μg/ml) of bovine collagen Type II in DPBS containing calcium and magnesium (DPBS++). The slides are then washed twice with cold DPBS++ and seeded with 8000 CHO-CCR5 or CHO pC4 transfected cells in a total volume of 125 μl and incubated at 37 °C in the presence of 95% oxygen / 5% carbon dioxide.

The culture medium is gently removed by aspiration and the adherent cells are washed twice with DPBS++ at ambient temperature. The slides are blocked with DPBS++ containing 0.2% BSA (blocker) at 0-4 °C for one hour. The blocking solution is gently removed by aspiration, and 125 μl of antibody containing solution (an antibody containing solution may be, for example, a hybridoma culture supernatant which is usually used undiluted, or serum/plasma which is usually diluted, e.g., a dilution of about 1/100 dilution). The slides are incubated for 1 hour at 0-4 °C. Antibody solutions are then gently removed by aspiration and the cells are washed five times with 400 μl of ice cold blocking solution. Next, 125 μl of 1 μg/ml rhodamine labeled secondary antibody (e.g., anti-human IgG) in blocker solution is added to the cells. Again, cells are incubated for 1 hour at 0-4 °C.

The secondary antibody solution is then gently removed by aspiration and the cells are washed three times with 400 μl of ice cold blocking solution, and five times with cold DPBS++. The cells are then fixed with 125 μl of 3.7% formaldehyde in DPBS++ for 15 minutes at ambient temperature. Thereafter, the cells are washed five

times with 400 µl of DPBS++ at ambient temperature. Finally, the cells are mounted in 50% aqueous glycerol and viewed in a fluorescence microscope using rhodamine filters.